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(54) Title: AIDS VIRUS GENE EXPRESSION

(57) Abstract

The *tat-3* gene of HTLV-III is expressed at high levels in *E. coli* and is reactive with antibodies induced in response to infection by HTLV-III and can induce production of antibodies which are reactive with HTLV-III.

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TITLE

AIDS Virus Gene Expression

FIELD OF THE INVENTION

This invention relates to the field of molecular biology and more particularly to expression of a gene from the HTLV-III virus in E. coli and uses thereof.

BACKGROUND OF THE INVENTION

Human T-lymphotropic virus type-III (HTLV-III), also known as Lymphadenopathy virus (LAV) or AIDS-associated retro-virus (ARV), is the etiological agent of the acquired immuno-deficiency syndrome (AIDS) and related disorders. While HTLV-III is evolutionarily more closely related to the ungulate lenti-retroviruses, it shares many common features with the previously isolated human T-lymphotropic viruses, types I and II (HTLV-I and HTLV-II), particularly those biological and pathogenic properties that are consequences of their capacity to infect helper T-lymphocytes and impair immune function. Furthermore, one unusual property unites HTLV types I, II and III, related animal retroviruses, such as bovine leukemia virus and simian T-lymphotropic viruses, type I, and the ungulate lenti-retroviruses, namely, the presence of a viral encoded protein which mediates activation of transcription initiated in the viral long terminal repeat (LTR). It has been speculated that this transcriptional activator (tat) plays a critical role in the biological

activities (transformation or cytopathic effects) of this group of viruses.

The severity of AIDS makes early and accurate diagnosis of infection by HTLV-III and detection and elimination of HTLV-III-contaminated samples from blood banks extremely important. Gallo et al., U.S. Patent 4,520,113, disclose use of antigens derived from HTLV-III to detect presence of anti-HTLV-III antibodies in serum. Montagnier et al., EP-A-138,667, disclose use of a specified HTLV-III antigen to detect infection by the virus. Papas et al., United States Patent Application Serial No. 6-664,972 (Derwent Accession No. 85-110268/18), disclose expression of a HTLV-I envelope protein coding sequence in E. coli and use of the protein expressed thereby to detect infection by HTLV-I. Crowl et al., Cell 41: 979(1985), report expression in E. coli of portions of the HTLV-III envelope protein gene, env, and use of such proteins for detection of infection by HTLV-III. Casey et al., J. Virol. 55: 417 (1985), report purification of the gag gene product, an internal structural protein of HTLV-III referred to as p24, and use of the protein to detect infection by HTLV-III.

Seiki et al., Proc. Nat'l. Acad. Sci USA 80: 2618 (1983), and Haseltine et al., Science 225: 419 (1984), report identification of proteins which mediate activation of transcription of the LTR in HTLV-I and HTLV-II, referred to as the tat-1 and tat-2 proteins, respectively.

Sodroski et al., Science 227: 171 (1985), report in trans activation of gene expression from the LTR in HTLV-III.

Arya et al., Science 229:69(1985), and Sodroski et al., Science 229: 74 (1985), report a tat protein encoded by HTLV-III and identification and cloning in E. coli of a cDNA coding for said tat protein, referred to as tat-3.

SUMMARY OF THE INVENTION

The invention is, in one aspect, an E. coli expression vector which comprises a DNA coding sequence operatively linked to a regulatory element wherein the DNA coding sequence codes for the tat-3 protein of HTLV-III or for a derivative thereof, which derivative is a polypeptide which is reactive with antisera to tat-3 induced in response to infection in an animal by HTLV-III.

In another aspect, the invention is a method for detecting infection in an animal by HTLV-III which comprises contacting a sample of serum from the animal with tat-3, or a derivative thereof which derivative is a polypeptide which is reactive with antisera to tat-3 induced in response to infection in an animal by HTLV-III, and assaying for reactivity of the sample with the tat-3 or the tat-3 derivative.

All of these embodiments of the invention, as well as others described herein, are readily attainable and are considered as further aspects of the same invention.

DETAILED DESCRIPTION OF THE INVENTION

It has now been discovered that the tat-3 protein of HTLV-III can be expressed in E. coli in readily recoverable quantities and that the protein so expressed is reactive with sera from animals infected by the HTLV-III virus.

The E. coli expression vector of the invention is prepared by recombinant DNA techniques or by a combination of recombinant DNA and synthetic techniques. It comprises at least a coding sequence for the tat-3 protein of HTLV-III or for a derivative of the tat-3 protein which is immunologically equivalent to tat-3. By "immunologically equivalent" is meant that the derivative polypeptide is reactive with antibodies to authentic tat-3 induced in

response to infection in an animal by HTLV-III or, conversely, is capable of inducing an immune response which is reactive with authentic tat-3. In the expression vector of the invention, said coding sequence is operatively linked to a regulatory element.

The coding sequence for authentic tat-3 can be prepared by known techniques from HTLV-III virus or from HTLV-III-infected cells by isolation of viral mRNA and preparing cDNA by reverse transcription. Such preparation of a tat-3 coding sequence is disclosed by Arya et al., Science 229: 69 (1985) and by Sodroski et al., Science 229: 74 (1985), both of which are incorporated by reference herein.

Derivatives of the coding sequence so obtained can be prepared by standard recombinant DNA and/or synthetic techniques. These include mutation techniques reviewed by Botstein, Science 229:1193 (1985). Such derivatives can comprise addition, substitution or deletion of one or more base pairs such that upon expression, the resulting fused, mutated or truncated polypeptide is immunologically equivalent to authentic tat-3. Typically, for use as a diagnostic, such derivative will comprise a truncated protein, for example a polypeptide of 5 to 10 amino acids which retains immunologic cross-reactivity with tat-3, such as a tat-3 protein in which N- or C-terminal amino acids have been deleted.

The coding sequence for the polypeptide can be inserted into any E. coli expression vector, many of which are known and available. By "regulatory element" is meant the expression control sequences, for example, a promoter and ribosome binding site, required for transcription and subsequent translation. Regulatable regulatory elements, that is, regulatory signals which are not constitutive but require induction or derepression, are preferred. Such vectors typically comprise, in addition to the regulatory

element, a region which permits the vector to be stably maintained in a host cell population, that is, a replicon or origin of replication, and one or more selection markers, that is, genes which confer a selectable phenotype upon hosts carrying the vector. One exemplary expression vector of the invention is the plasmid pAS1, described by Rosenberg et al., Meth. Enzym., 101: 123 (1983) and Shatzman et al., in Experimental Manipulation of Gene Expression, edit. by M. Inouye, Academic Press, New York, 1982. pAS1 carries the pBR322 origin of replication, an ampicillin resistance marker and a series of fragments from bacteriophage lambda, which comprise the regulatory element including PL, N anti-termination function recognition sites (NutL and NutR), the rho-dependent transcription termination signal (tR1) and the cII ribosome binding site (rbs), including the cII translation initiation site, the G residue of which is followed immediately by a BamHI cleavage site as follows:

5'...cII...CA!TATG*GATCC...3'

wherein the symbol, *, indicates the clevage site for BamHI and the symbol,!, indicates the cleavage site for NdeI.

pAS1 can be derived from pKC30cII by deleting nucleotides between the BamHI site at the lambda-pBR322 junction of pKC30cII and the cII ATG and religating the molecule to regenerate the BamHI site immediately downstream of the ATG. pKC30cII is constructed by inserting a 1.3 kb HaeIII fragment from lambda which carries the cII gene into the HpaI site of pKC30. See Shatzman et al., cited above, and Rosenberg et al., cited above. pKC30 is described by Shimatake et al., Nature,

292: 128 (1981). It is a pBR322 derivative having a 2.4 kb HindIII-BamHI fragment of lambda inserted between the HindIII and BamHI sites in the tetR gene of pBR322. Constructions similar to pAS1 are described by Courtney et al., Nature, 313, 145 (1985) and Kotewicz et al., Gene 35: 249(1985). Derivatives of pAS1, comprising the PL, NutL, NutR and cII rbs regulatory element, can be constructed by standard techniques. The coding sequence is operatively linked, that is, in correct orientation and in proper reading frame, to a regulatory element of an E. coli expression vector by standard techniques to construct an expression vector of the invention.

The tat-3 expressed by E. coli, as shown in the Examples below, was reactive with 42 of 92 samples of sera (46%) from individuals exposed to HTLV-III, but was not reactive with sera from normal individuals. Thus, tat-3 and derivatives thereof can be used in detection of HTLV-III infection by standard assay techniques which permit detection of presence of tat-3 protein or anti-tat-3 antibodies. The known host range for HTLV-III is limited to man and certain other higher primates, although presence in a larger animal pool at this time or in the future cannot be ruled out. Preferably, tat-3 is used in a battery of one or more other tests, such as immunoassays for presence of the env, sor, gag or 3'orf gene products in sera. Based on data gathered to date, a positive reaction with tat-3 is 100% diagnostic of HTLV-III infection. E. coli-derived tat-3 can also be used to screen samples of blood in blood banks.

Techniques for employing tat-3 in such diagnostic immunoassays are well known. These include, for example, the technique disclosed by Casey et al., J. Virol. 55: 417 (1985) and by Crowl et al., Cell 41: 979 (1985). tat 3 can be employed in an enzyme linked immunosorbent assay

(ELISA) or radioimmuno assay (RIA). Preferably, a western blotting assay is employed, as an ELISA assay has been found in preliminary experiments to result in a small percentage of false positives.

Also, the tat-3 protein produced by E. coli can be used to stimulate production of anti-sera which is reactive with HTLV-III. Thus the tat-3 protein can be used as an antigenic component of a vaccine against infection by HTLV-III, although the protein is not structural and appears to be localized in cell nuclei. The ability to raise polyclonal antibodies renders it possible also to produce monoclonal antibodies by the standard techniques originally described by Kohler and Milstein, Nature 256:495 (1975) or other techniques of cell fusion or transformation. Such polyclonal or monoclonal antibodies can also be useful in detecting presence of tat-3 gene product in sera or in a cell population such as a cell culture. Such antibodies are also useful in affinity purification of tat-3, in epitope mapping to localize functional domains within the protein, such as domains which function in DNA binding, and can be used as neutralizing agents in therapy for HTLV-III infection. The tat-3 gene product can also be used in regulation of LTR-controlled gene expression units as disclosed by Arya et al. and Sodroski et al., et al, cited above. Because tat 3 is, by definition, functional in trans, an expression vector of the invention can be used to control expression for an integrated gene expression unit or a gene expression unit present in another plasmid.

The predicted amino acid sequence of the tat-3 gene product (see, Example 1, below) reveals a highly hydrophilic protein with at least three discernible domains in the first 57 amino acids: a proline-rich region (5 out of 16 residues from positions 2-18), a cysteine-rich region (7 out of 16 residues from positions 22-37) and a lysine/arginine-rich region (8 out of 9

residues from positions 49-57). The availability of highly purified tat-3 protein in sufficient quantities will allow direct elucidation of its sites(s) and mechanism(s) of action, e.g., its DNA binding properties. In addition, expression of various truncated and mutated forms of this protein will allow precise localization of its functional domains. Availability of E. coli-derived tat-3 will permit identification of effectors, especially inhibitors of tat-3 function which can be used in therapy for HTLV-III infection.

Although the tat-3 gene of HTLV-III may be functionally analogous to the tat-1 and tat-2 genes and all three are transcribed from three exons into an RNA of about 2 kilobases, tat-3 differs from tat-1 and -2 including, for example, in its position in the genome, its size and its primary nucleotide sequence.

The following Examples are illustrative, and not limiting, of the invention and of techniques for making and using the invention.

Example 1

pOTS34 was derived from pAS1 by inserting a 189 bp fragment carrying a transcription terminator, the oop terminator, into the NruI site downstream of the BamHI site in pAS1 and by inserting a synthetic linker (XbaI, XhoI, SacI) into the SalI site between the BamHI site and the added terminator. pOTS34 is identical to pOTS-5 or pOTSV (Devare et al., Cell 36: 43 (1984)) except that the linker is inserted in opposite orientation.

A vector carrying a cDNA for tat-3, pCV-1, (Arya et al., cited above) was employed as a source of the tat-3 coding sequence. The nucleotide and predicted amino acid sequence of the cDNA in pCV-1 are as follows:

```
1  ATG GAG CCA GTA GAT CCT AGA CTA GAG CCC TGG AAG CAT CCA GGA AGT CAG CCT AAA ACT
   MET GLU PRO VAL ASP PRO ARG LEU GLU PRO TRP LYS HIS PRO GLY SER GLN PRO LYS THR

   GCT TGT ACC AAT TGC TAT TGT AAA AAG TGT TGC TTT CAT TGC CAA GTT TGT TTC ATA ACA
   ALA CYS THR ASN CYS TYR CYS LYS LYS CYS CYS PHE HIS CYS GLN VAL CYS PHE ILE THR

5  AAA GCC TTA GGC ATC TCC TAT GGC AGG AAG AAG CGG AGA CAG CGA CGA AGA CCT CCT CAA
   LYS ALA LEU GLY ILE SER TYR GLY ARG LYS LYS ARG ARG GLN ARG ARG ARG PRO PRO GLN

   GGC AGT CAG ACT CAT CAA GTT TCT CTA TCA AAG CAA CCC ACC TCC CAA TCC CGA GGG GAC
   GLY SER GLN THR HIS GLN VAL SER LEU SER LYS GLN PRO THR SER GLN SER ARG GLY ASP

   CCG ACA GGC CCG AAG GAA TAG
10 PRO THR GLY PRO LYS GLU END
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The strategy used to express the complete tat-3 protein involved two stages. First, the tat-3 coding region lacking the first 12 base-pairs (bp) at its 5' end was obtained as a MboI restriction endonuclease fragment from the cDNA clone. This fragment was inserted at the BamHI site of the pOTS34 vector. The resultant construct, pOTS-tatIIID, contains the tat-3 coding sequence deleted in codons 2 to 4 and positioned in-frame with the initiation codon provided by pOTS34. The second stage involved regeneration of the three missing codons at the amino-terminus. Since the 5' but not the 3' BamHI site was recreated in the pOTS-tatIIID plasmid, this vector was digested with BamHI, followed by Mung Bean exonuclease to create a blunt-end cloning site immediately adjacent to the initiation codon and the fifth codon of tat-3. A synthetic DNA linker reconstructing the missing codons was then inserted. The nucleotide sequence of the linker was slightly modified from the tat-3 gene without altering the amino acid sequence such that the 2d, 3d and 4th codons were as follows: GAA CCG GTG. This construction resulted in a BamHI site between the 4th and 5th codons. The final construction, pOTS-tatIII, consists of the reconstructed full-length tat-3 coding sequence in

frame with the ATG of pOTS34. A sample of pOTS-tatIII has been deposited under the terms of the Budapest Treaty in the American Type Culture Collection, Rockville, Maryland, under Accession Number 53305.

pOTS-tatIIID, pOTS-tatIII and a control vector without insert (pOTS34), were introduced into E. coli AR120, a cI^+ lysogen inducible by nalidixic acid. See Mott et al., Proc. Nat'l. Acad. Sci. USA 82:88 (1985). Aliquots of the bacterial lysate at different times after induction were subjected to polyacrylamide gel electrophoresis. The AR120 bacterial cells containing pOTS34, pOTS-tatIIID or pOTS-tatIII were grown to OD650 = 0.4-0.5 and induced by the addition of nalidixic acid to 60 µg/ml substantially as described by Mott et al., cited above Aliquots were taken 0, 3, and 5 hours after induction, spun down and resuspended in lysis buffer (60mM Tris-HCl (pH 7.0), 10% glycerol, 5% 2-mercaptoethanol, 2% SDS, 0.1% bromophenolblue). The proteins were resolved on a 15% SDS-polyacrylamide gel (acrylamide:bisacrylamide ratio of 30:0.8) and visualized by staining with Coomassie Brilliant Blue R-250.

A protein migrating with a 14 kd lysozyme marker was specifically induced in the cells transfected with pOTS-tatIIID, and a slightly larger protein was detected in the cells transfected with pOTS-tatIII. Although the apparent molecular size of 14 kd is greater than that expected for tat-3, about 9.7 kd based on the amino acid sequence, the discrepancy is attributed to the high proline content of this protein which could have retarded its migration. This example demonstrates high level expression of the tat-3 coding sequence in E. coli.

Example 2

The production of highly expressed tat-3 protein in bacteria (2-5% of total cellular protein), as in Example 1, allowed preparation of specific polyclonal antibodies

against it. For this purpose the *E. coli*-derived *tat-3* protein, purified by electroelution following resolution on preparative polyacrylamide gels, was injected subscapularly into New Zealand white rabbits. Antisera from immunized rabbits reacted well against the 14 kd protein expressed in bacteria. Furthermore, a similar sized protein, 14 kd by polyacrylamide gel electrophoresis, was detected at low levels in an infected T-lymphocyte cell line (H9/HTLV-III-B) by immunoprecipitation. This latter data was the first evidence that *E. coli*-derived *tat-3*, even after SDS gel electrophoresis, retained epitopes in common with native *tat-3*, thus demonstrating that bacterially-derived *tat-3* can be used to produce antibodies which are reactive with authentic *tat-3*.

Example 3

To evaluate whether the *tat-3* protein could be of diagnostic or prognostic value, sera from diverse individuals were examined for reactivity against the partially purified protein by Western blotting. Specifically, following partial purification, resolution on SDS-polyacrylamide gel and electrotransfer to a nitrocellulose membrane, the membrane was cut into strips such that each strip was estimated to contain 1ug of *tat-3*. The strips were incubated at room temperature for 1 hr in milk buffer (5% non-fat dry milk, 0.1% Antifoam A, 0.1% N_aN_3, 0.9% NaCl) and then were incubated at 4°C with a 1/100 dilution of patient sera. The strips were then washed for 20 min in phosphate buffered saline (PBS) and incubated for 1 hr at room temperature (20-25°C) in the milk buffer containing ^{125}I- labelled protein A. After three washes for 30 min each in PBS, the strips were dried and autoradiographed. A 14.3 kd band corresponded to *tat-3*. The results, as

summarized in Table 1 below, indicate that while all healthy normal people not known to be exposed to HTLV-III lacked antibodies to tat-3, a substantial fraction of people who were seropositive for other viral structural proteins (envelope and core antigens) had detectable antibody level to tat-3.

One hundred seven serum samples were examined. They were divided into four categories, based on accepted Center for Disease Control definitions: (1) healthy seronegative (no reactivity with gag or env proteins) (HN); (2) healthy HTLV-III carriers (includes sera from individuals in high risk populations and positive for HTLV-III antibodies against env or gag protein, but free of clinical symptoms) (HC), (3) individuals with AIDS-related complex (ARC); and (4) individuals with acquired immuno deficiency syndrome (AIDS).

None of the serum samples from category (1) were reactive with the tat-3. Approximately similar percentages of samples (53%, 29% and 53%) in each of the other categories were reactive with the tat-3. Reactions ranged from strongly reactive to weakly reactive without correlation to the stage of progression of the disease.

Table 1

Sera	No. tested	No. positive	% positive
HN	15	0	0
HC	30	16	53
ARC	28	8	29
AIDS	34	18	53

The lower immunoreactivity of tat-3 as compared to env or gag proteins may be due to the lower level of the

protein expressed *in vivo*, presence of fewer immunogenic epitopes and the presumed nuclear localization of *tat-3*. This example demonstrates utility of the bacterially-derived *tat-3* protein in diagnosing infection by HTLV-III in an animal.

The above description and Examples are illustrative of the invention and of preferred embodiments thereof. The invention, however, is not limited to embodiments specifically disclosed herein but rather includes all modifications coming within the scope of the claims which follow.

Claims:

1. An E. coli expression vector which comprises a DNA coding sequence operatively linked to a regulatory element wherein the DNA coding sequence codes for the tat-3 protein of HTLV-III or for a derivative thereof, which derivative is a polypeptide which is reactive with antisera to tat-3 induced in response to infection in an animal by HTLV-III.

2. The vector of claim 1 wherein the DNA coding sequence codes for a polypeptide having the amino acid sequence: N-MET (GLU PRO VAL)$_n$ ASP PRO ARG LEU GLU PRO TRP LYS HIS PRO GLY SER GLN PRO LYS THR ALA CYS THR ASN CYS TYR CYS LYS LYS CYS CYS PHE HIS CYS GLN VAL CYS PHE ILE THR LYS ALA LEU GLY ILE SER TYR GLY ARG LYS LYS ARG ARG GLN ARG ARG ARG PRO PRO GLN GLY SER GLN THR HIS GLN VAL SER LEU SER LYS GLN PRO THR SER GLN SER ARG GLY ASP PRO THR GLY PRO LYS GLU-C, wherein n is 0 or 1.

3. The vector of claim 2 wherein the DNA coding sequence is as follows: ATG (GAX CCX GTX)$_n$ GAT CCT AGA CTA GAG CCC TGG AAG CAT CCA GGA AGT CAG CCT AAA ACT GCT TGT ACC AAT TGC TAT TGT AAA AAG TGT TGC TTT CAT TGC CAA GTT TGT TTC ATA ACA AAA GCC TTA GGC ATC TCC TAT GGC AGG AAG AAG CGG AGA CAG CGA CGA AGA CCT CCT CAA GGC AGT CAG ACT CAT CAA GTT TCT CTA TCA AAG CAA CCC ACC TCC CAA TCC CGA GGG GAC CCG ACA GGC CCG AAG GAA TAG, wherein n is 0 or 1 and X is G or A.

4. The vector of claim 1, 2 or 3 wherein the regulatory element comprises the PL promoter of lambda, the Nut L and Nut R recognition sites and the cII ribosome binding site.

5. The vector of claim 1, 2 or 3 which is pAS1, or a derivative thereof, into which the coding sequence for tat-3 has been inserted.

6. The vector of claim 5 which is pOTS-tatIIID or pOTS-tatIII.

7. A method for detecting infection in an animal by HTLV-III which comprises contacting a sample of serum from the animal with tat-3, or a derivative thereof which derivative is a polypeptide which is reactive with antisera to tat-3 induced in response to infection in an animal by HTLV-III, and assaying for reactivity of the sample with the tat-3 or tat-3 derivative.

8. The method of claim 7 wherein the tat-3 or tat-3 derivative is derived from E. coli transformed with an expression vector which comprises a DNA coding sequence operatively linked to a regulatory element wherein the DNA coding sequence codes for the tat-3 protein of HTLV-III or for a derivative thereof, which derivative is a polypeptide which is reactive with antisera to tat-3 induced in response to infection in an animal by HTLV-III.

9. The method of claim 8 wherein the tat-3 or tat-3 derivative has the following amino acid sequence: N-MET $(GLU.\ PRO\ VAL)_n$ ASP PRO ARG LEU GLU PRO TRP LYS HIS PRO GLY SER GLN PRO LYS THR ALA CYS THR ASN CYS TYR CYS LYS LYS CYS CYS PHE HIS CYS GLN VAL CYS PHE ILE THR LYS ALA LEU GLY ILE SER TYR GLY ARG LYS LYS ARG ARG GLN ARG ARG ARG PRO PRO GLN GLY SER GLN THR HIS GLN VAL SER LEU SER LYS GLN PRO THR SER GLN SER ARG GLY ASP PRO THR GLY PRO LYS GLU-C, wherein n is 0 or 1.

10. The method of claim 9 wherein the DNA coding sequence is as follows: ATG $(GAX\ CCX\ GTX)_n$ GAT CCT AGA CTA GAG CCC TGG AAG CAT CCA GGA AGT CAG CCT AAA ACT GCT TGT ACC AAT TGC TAT TGT AAA AAG TGT TGC TTT CAT TGC CAA GTT TGT TTC ATA ACA AAA GCC TTA GGC ATC TCC TAT GGC AGG AAG AAG CGG AGA CAG CGA CGA AGA CCT CCT CAA GGC AGT CAG ACT CAT CAA GTT TCT CTA TCA AAG CAA CCC ACC TCC CAA TCC

CGA GGG GAC CCG ACA GGC CCG AAG GAA TAG, wherein n is 0 or 1 and X is G or A.

11. The method of claim 8, 9 or 10 wherein, in the vector, the regulatory element comprises the PL promoter of lambda, Nut L and Nut R recognition sites and the cII ribosome binding site.

12. The method of claim 8, 9 or 10 wherein the vector is pAS1, or a derivative thereof, into which the tat-3 coding sequence has been inverted.

13. The method of claim 12 in which the vector is pOTS-tatIIID or pOTS-tatIII.

14. The method of claim 7, 8, 9 or 10 which also comprises contacting the sample with one or more other HTLV-III gene products.

15. The method of claim 7, 8, 9 or 10 in which the sample is contacted with the tat-3 in a western blotting assay.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 86/02374

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4) C07/k 13/00, 15/04, C12N 7/00, 15/00; C12P19/34;
A61K39/12, 39/21 US 435/5, 68, 424/89 530/300

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
US	435/5, 6, 68, 70, 91, 253, 172.3, 317; 536/27, 935/29,81 424/85, 86, 89; 514/2, 12; 530/300, S26

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

CHEMICAL ABSTRACTS DATA BASE (CAS) 1967-1986
BIOLOGICAL ABSTRACTS DATA BASE (BIOSIS) 1969-1986
KEYWORDS: HTLV-III; LAV; ARV; TAT-III

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	SCIENCE, (Washington, D.C., U.S.A.), Volume 229, issued 05 July, 1985, (ARYA ET AL), "Trans-Activator Gene of Human T-Lymphotropic Virus Type III (HTLV-II), see page 69.	1-15
Y	SCIENCE, (Washington, D.C., U.S.A.), Volume 229, issued 05 July, 1985, (SODROSKI ET AL), "Location of the Trans-Ativating Region on the Genome of Human T-Cell Lymphotropic Virus Type III", see page 74.	1-15
Y	US,A 4520113 (GALLO ET AL), 28 May, 1985, see column 1,	1-15
Y,P	CELL, (Cambridge, Massachusets), Volume 43, issued December, 1985, (CROWL ET AL), "HTLV-III env Gene Products Synthesized in E. coli Are Recognized by Antibodies Present in the Sera of Aids Patients", see page 461.	7-15

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

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IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
23 DECEMBER 1986	05 JAN 1987
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	Stephanie Seidman Ph.D. J.D.

Form PCT/ISA/210 (second sheet) (October 1981)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	PROCEEDING NATIONAL ACADEMY SCIENCES (Washington, D.C., U.S.A.), Volume 81, issued October 1984, (KIYOKAWA ET AL), "Envelope proteins of human T-cell leukemia virus: Expression in Escherichia coli and its application to studies of env gene functions", see page 6202.	1-15

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers....., because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
Y	PROCEEDINGS NATIONAL ACADEMY SCIENCES (Washington, D.C., U.S.A.), Volume 82, issued November, 1985, (DOWBENKO ET AL), "Bacterial expression of the acquired immunodeficiency syndrome retrovirus p24 gag protein and its use as a diagnostic reagent", see page 7748.	1-15